Cyclic-di-guanosine monophosphate or c-di-GMP is a ubiquitous bacterial second messenger. It is synthesized by diguanylate cyclases and hydrolyzed by cognate phosphodiesterases. A state of balance in these two opposing activities determines the healthy cellular concentrations of c-di-GMP. Depending upon concentration, it regulates a variety of cellular processes. These phenotypes include transition between motility and sessility, virulence, biofilm formation, long-term survival and cell differentiation among many others.

Chapter 1 introduces our basic and current understanding of c-di-GMP signaling pathway in brief in the bacterial kingdom. We have summarized recent progress in this regards. Special attention has been given to the methodological advancements to study c-di-GMP signaling and polymorphism of c-di-GMP itself. A careful study of polymorphism of c-di-GMP motivated us to investigate this aspect in detail. The data obtained in this analysis and our current understanding about polymorphism of c-di-GMP prompted us to propose that polymorphism of c-di-GMP can present itself as a layer of control in the c-di-GMP signaling pathways.

Chapter 2 deals with the synthesis of a fluorescent analog of c-di-GMP named as MANT-(c-di-GMP) or MANT-CDG. We have followed a well established protocol to synthesize MANT-CDG. It was then purified and validated using chromatographic methods and mass spectrometry. Further, various physico-chemical and spectral characterizations have been carried out. We noticed that upon excitation at 355 nm, it shows a significant emission with maxima at ~440 nm. It also exhibits several properties of an ideal probe, most importantly; its fluorescence is sensitive to change in microenvironment.

Chapter 3 details the biochemical characterization of MANT-CDG. Because fluorescence of MANT-CDG is sensitive to change in microenvironment, we wanted to establish whether it can be used as a probe to study interaction with c-di-GMP metabolizing proteins. Interestingly, we observed that it binds with a diguanylate cyclase, a phosphodiesterase and a receptor protein in a fashion similar to the native c-di-GMP. We also noticed that it can be used as a substrate to monitor real time activity of a phosphodiesterase.

Chapter 4 deals with the biochemical characterization of DcpA from *Mycobacterium smegmatis*. It is a multidomain protein having GAF-GGDEF-EAL domains arranged in tandem. It shows both the c-di-GMP synthesis as well as hydrolysis activities. We have studies the effect

of metals, GTP and c-di-GMP on the structure, activity and oligomerization of DcpA. Further, oligomerization studies on DcpA have been performed in details. We observed that DcpA exists in monomeric and dimeric forms. These forms are inter-convertible and two protomers are aligned parallel in a dimer.

Chapter 5 describes the biochemical analyses to show that the dimer possesses both activities i.e. synthesis and hydrolysis of c-di-GMP whereas the monomer shows only hydrolysis activity. We also observed that DcpA localizes to the inner membrane mostly at poles. An altered level of DcpA was found to affect the change in cell length, viability and colony morphology of *M. smegmatis*.

In **chapter 6**, we have briefly summarized the entire work carried out in this thesis.